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Colony Growth and Morphological Characterization of Sugarcane Root Bacteria and Sugarcane Field Exploration Bacteria in Fermented Liquid

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Abstract Rhizosphere bacteria play crucial roles in sugarcane fertility by providing nutrients, protecting against pathogens, and producing growth hormones. Exploring these bacteria can enhance sugarcane productivity through biofertilizers, biostimulants, and bioprotectants, reducing the need for costly inorganic fertilizers. This exploration is vital for optimizing sugarcane cultivation. The aims of the study were to determine the growth of bacterial colonies over 1, 2, and 3 weeks and to identify the morphology of sugarcane root bacteria and sugarcane exploratory bacteria growing in fermented liquid. The results showed: (i) Growth in the number of sugarcane root bacterial colonies was 7.7×10^7 CFU/ml in week 1, increasing to 28.8×10^7 CFU/ml in week 2, and further to 58×10^7 CFU/ml in week 3 in fermented liquid. The growth of bacterial colonies exploratory of sugarcane land was 3.4×10^7 CFU/ml in week 1, increasing to 19.7×10^7 CFU/ml in week 2, and further to 62×10^7 CFU/ml in week 3 in fermented liquid; (ii) Morphology of sugarcane root bacterial colonies from week 1 to week 3 included colony colors: yellow, pink, blue, milky white, blue center; colony sizes: pinpoint, medium, large; colony shapes: circular, irregular; elevation: convex, flat; surface textures: smooth, mucoid; edges: regular, irregular. Bacterial cell shape was bacilli and Gram-positive; and (iii) The morphology of bacterial colonies from sugarcane field exploration from week 1 to week 3 included colony colors: yellow,

pink, blue, milky white, blue center; colony sizes: small, medium, large, pinpoint; colony shapes: circular, irregular; elevation: convex, flat, umbonate, raised; surface textures: smooth, mucoid; edges: regular, irregular.

Keywords: Sugarcane, Root, Field, Bacteria, Fermented liquid

INTRODUCTION

Sugarcane is a sugar-producing plant and a significant source of carbohydrates. Its demand continues to increase along with the growing population. Sugarcane is the primary source of sweeteners globally, with almost 70% of sweeteners derived from it, while the rest come from sugar beets. In 2021, sugarcane production in Indonesia reached 2.42 million tonnes, with East Java producing 1.12 million tonnes (Mahdi, 2022).

One of the factors affecting the fertility of sugarcane is the presence of rhizosphere bacteria. Rhizospheric bacteria are those that live around the root area, possessing the ability to colonize roots and playing a crucial role in plant growth (Ashrafuzzaman et al., 2009). The use of rhizosphere bacteria as biological fertilizers is a biotechnological approach to increasing agricultural productivity. These bacteria can enhance plant growth by synthesizing and regulating various growth regulators, facilitating the availability of essential nutrients, and controlling soil pathogens (bioprotectants) (Marom et al., 2017).

Bacterial populations in the rhizosphere are generally more numerous and diverse than those in non-rhizosphere soils (Niswati et al., 2008). The activity of rhizosphere bacteria is influenced by exudates produced by plant roots. Some rhizosphere microorganisms play roles in nutrient cycling, soil formation, plant growth, bacterial activity, and biological control against root pathogens (Simatupang, 2008).

Rhizospheric bacteria have various roles, such as providing nutrients for plants, protecting plants from pathogenic bacterial infections (especially in the root area), and producing growth hormones like indole acetic acid, phosphate solvents, and nitrogen fixers. Additionally, these bacteria can affect the availability and cycling of plant nutrients by maintaining soil structure stability.

According to Saraswati et al. (2008), soil microbes function in four main ways: providing nutrients in the soil, decomposing organic matter and aiding in organic mineralization, spurring plant growth, and acting as biological agents controlling pests and diseases. These microbial roles also influence the chemical and physical properties of soil and plant growth. Saraswati (2006) explained that knowing the population size and activity of microbes in soil can indicate soil fertility because a high microbial population signifies sufficient organic matter, suitable temperature, adequate water availability, and favorable soil ecological conditions.

Estimations show that the number of bacteria by direct count is 2 billion cells per gram of soil, constituting only 0.2 percent of the soil's weight. This amount is equivalent to 4,480 kilograms of bacterial biomass per hectare of soil at a depth of 15 cm. Direct counts of bacterial populations range from several hundred thousand to two hundred million bacteria per gram of dry soil (Susilawati et al., 2016).

According to Morales-García et al. (2011), rhizosphere bacteria stimulate plant growth by producing various growth regulators in the root environment, regulating the balance of hormones and nutrients, enhancing resistance to plant pathogens, and dissolving nutrients to facilitate their absorption by plants. Rhizospheric bacteria can interact both compatibly and antagonistically with microorganisms inside and outside the rhizosphere, indirectly promoting plant growth (Vejan et al., 2016).

The roles of plant rhizosphere bacteria can be categorized as biofertilizers (increasing the availability of plant nutrients), biostimulants (producing phytohormones), rhizomediators (reducing the amount of organic pollutants in the soil), and biopesticides (controlling diseases by producing antibiotics and antifungal metabolites) (Ahemad & Kibret, 2014).

The application of rhizosphere and soil bacteria as biological fertilizers is an alternative method to enhance soil fertility and reduce environmental pollution caused by excessive use of inorganic fertilizers. These bacteria can increase plant growth through mechanisms like nitrogen fixation, phosphate solubilization, and hormone production (indole acetic acid). The use of rhizosphere bacteria can reduce the need for organic fertilizers by 25-50%, phosphate solubilization by 50%, and hormone production by 50% (Jannah et al., 2022).

Bacterial growth is influenced by several factors, including medium, nutrition, temperature, oxygen, pH, and environment. The nutrients required for bacterial growth include sources of carbon, nitrogen, amino acids, and vitamins. Adequate nutrients, in appropriate amounts, enhance bacterial growth. Light and humidity significantly affect bacterial growth, with bacteria generally requiring high humidity (± 85%). Reducing the water content of protoplasm can halt metabolic activities, as seen in the processes of freezing and drying bacterial cells (Dwidjoseputro, 1998).

Given the various capabilities and roles of rhizosphere bacteria, it is essential to explore these bacteria in sugarcane plants. Identifying beneficial rhizosphere bacteria can lead to their optimal utilization in sugarcane plantations as biofertilizers, biostimulants, and bioprotectants. This exploration is particularly important due to the rising costs of inorganic fertilizers and the need to boost sugarcane productivity. The aims of this study were to determine the growth of bacterial colonies over 1, 2, and 3 weeks and to identify the morphology of sugarcane root bacteria and sugarcane exploratory bacteria growing in fermented liquid.

METHOD

Sugarcane Root Bacteria and Sugarcane Exploration Bacteria in Fermented Liquid

To prepare the fermented liquid, shrimp paste is added to boiling water, followed by molasses, and stirred. Potatoes are added to the water in the pot, and then separated to obtain the potato extract solution. Corn bran is placed into a bucket, tightly closed, and mixed with the shrimp paste and molasses solution along with the potato extract solution, then stirred until evenly distributed and the bucket is closed tightly. For the preparation of PDA media for inoculating bacteria from sugarcane roots, the roots are cut into small pieces and placed into the PDA medium. Pineapple pieces are blended with water, and the resulting mixture is added to the PDA solution, stirred until evenly distributed, and closed tightly. For bacteria exploration in sugarcane fields, soil samples from sugarcane fields are added to the PDA medium. Pineapple pieces are blended with water, and the mixture is added to the PDA solution, stirred until evenly distributed, and closed tightly. The development of sugarcane exploratory bacteria is then observed.

Total Bacterial Colonies

Sample dilution is carried out in multiples of 1:10, with each dilution suspension inoculated using the pour plate method. After incubation for 18-24 hours, bacterial colonies form on the agar plates. The colonies are counted using a colony counter equipped with an electronic recorder, only live bacteria are counted. To ensure this, the bacterial medium is diluted, for example, up to 3 times in a test tube, then planted and incubated, and the growing colonies are counted.

Morphological Characterization of Bacterial Colonies

Macroscopic observations of the bacterial liquid colonies are made by observing the shape and color. Three types of observations are performed: from above to determine the overall shape of the colony, from above to determine the shape of the colony edge, and from the side to determine the height of the colony.

Gram Stain Technique

For the Gram stain technique, distilled water is placed on a glass slide, and 1 ose of sample culture is added and fixed by passing it over a flame. Crystal violet stain is applied and left for 1 minute, then rinsed with running water. Lugol's iodine is then applied, left for 1 minute, and rinsed again. The slide is decolorized with 96% alcohol for 10-20 seconds, rinsed with water, and then safranin stain is applied and left for 20-30 seconds before a final rinse. The slide is dried with absorbent paper, immersion oil is added, and it is observed under a microscope. Gram-negative bacteria stain red, while gram-positive bacteria stain purple.

FINDINGS AND DISCUSSION

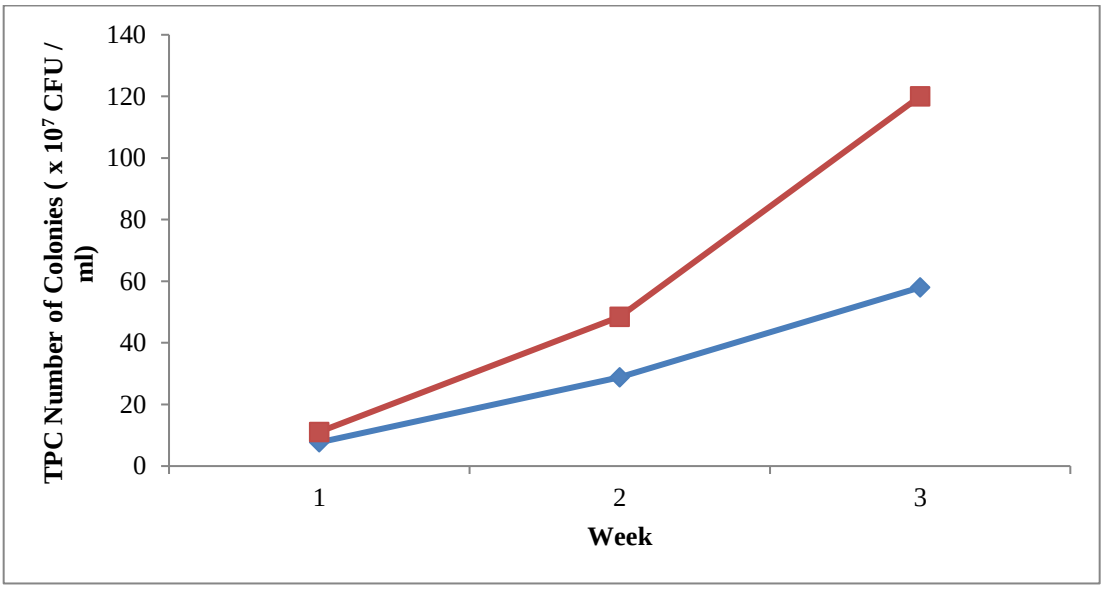
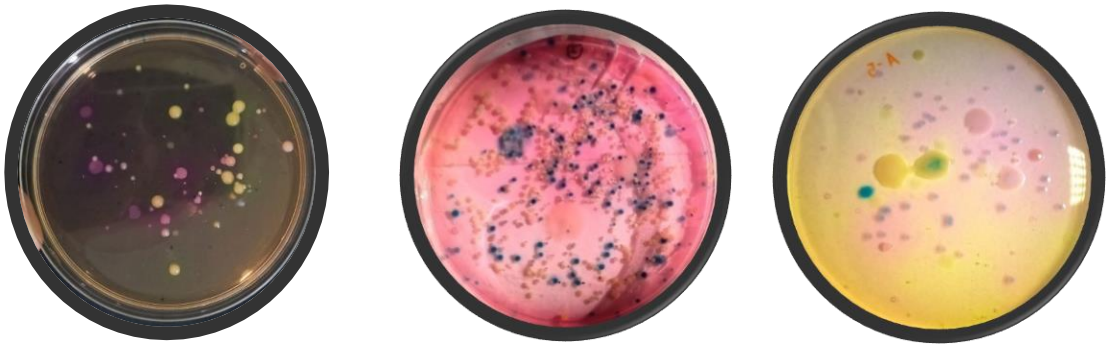


Figure 1. Development of sugar cane root bacterial colonies (blue line) and sugarcane field exploration bacteria (red line).

Figure 1 shows the growth of bacterial colonies in sugarcane root bacteria and sugarcane exploratory bacteria in fermented liquid. The number of sugarcane root bacterial colonies was 7.7 × 10^7 CFU/ml in week 1, which increased to 28.8 × 10^7 CFU/ml in week 2 and further increased to 58 × 10^7 CFU/ml in week 3. Similarly, the number of exploratory bacterial colonies in sugarcane land was 3.4 × 10^7 CFU/ml in week 1, which increased to 19.7 × 10^7 CFU/ml in week 2 and further increased to 62 × 10^7 CFU/ml in week 3. This indicates that the bacteria continued to grow, suggesting that the nutrients and the medium still supported bacterial growth.



Week 1 (A) Week 2 (B) Week 3 (C)

Figure 2. Development of sugar cane root bacterial colonies (A, B, C).

Table 1. Morphology of sugarcane root bacterial colonies week 1.

ISOLATE CODE	COLONY MACROSCOPY					
	Colony Color	**Size**	**Colony Form**	**Elevation**	**Surface**	**Edge**
1.	Yellow	Big	Circular	Convex	Mucoid	Regular
2.	Pink	Big	Irregular	Flat	Smooth	Irregular
3.	Blue	Pints / Points	Circular	Convex	Smooth	Regular

Table 2. Morphology of sugarcane root bacterial cells week 1.

ISOLATE CODE	CELL MORPHOLOGY	
	Cell Shape	**Grams**
1.	Basil	Positive
2.	Basil	Positive
3.	Basil	Positive

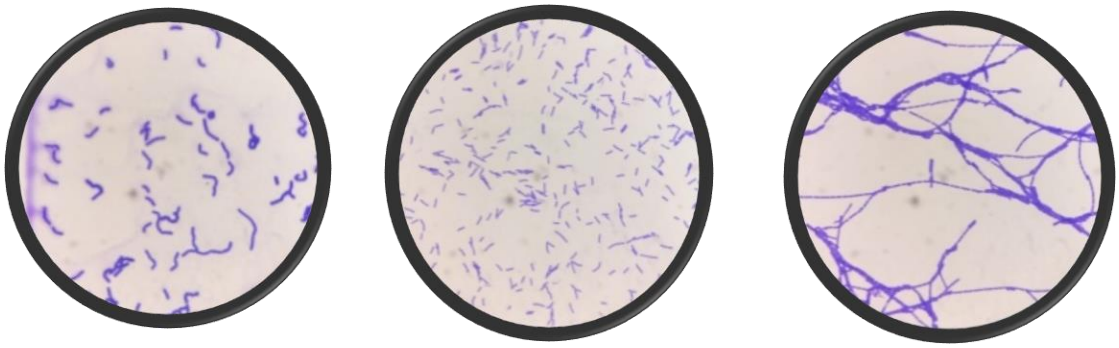


Figure 3. Results of gram staining of sugarcane root bacteria week 1.

Based on the morphological observations of sugarcane root bacterial colonies in week 1, three distinct colony colors were observed: yellow, pink, and blue. The characteristics of each colony are as follows:

1. **Yellow Colony**:
   - Size: Large
   - Shape: Circular (round with edges)
   - Elevation: Convex
   - Texture: Mucoid
   - Edges: Regular
2. **Pink Colony**:
   - Size: Large
   - Shape: Irregular (with uneven edges)
   - Elevation: Flat
   - Surface: Flat
   - Edges: Irregular
3. **Blue Colony**:
   - Size: Point

- Shape: Circular
- Elevation: Convex
- Surface: Flat
- Edges: Regular
- Cell Shape: Bacilli and gram-positive

Table 3. Morphology of sugarcane root bacterial colonies week 2.

ISOLATE CODE	COLONY MACROSCOPY					
	Colony Color	**Size**	**Colony Form**	**Elevation**	**Surface**	**Edge**
1.	Blue	Medium	Circular	Convex	Smooth	Regular
2.	Milk White	Medium	Circular	Convex	Mucoid	Regular
3.	Pink	Big	Circular	Flat	Mucoid	Irregular

Table 4. Morphology of sugarcane root bacterial cells week 2.

ISOLATE CODE	CELL MORPHOLOGY	
	Cell Shape	**Grams**
1.	Basil	Positive
2.	Basil	Positive
3.	Basil	Positive

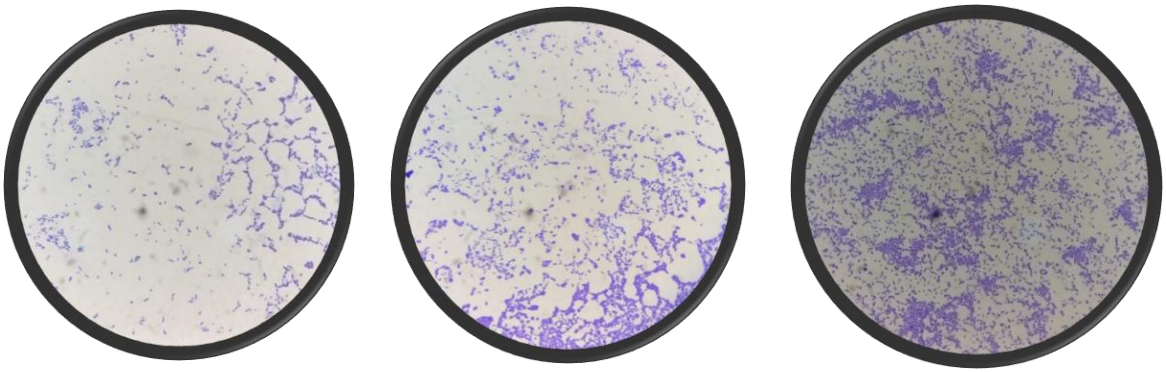


Figure 4. Results of gram staining of sugarcane root bacteria week 2.

Based on the morphological observations of sugarcane root bacterial colonies in week 2, three distinct colony colors were observed: blue, milky white, and pink. The characteristics of each colony are as follows:

1. **Blue Colony**:
   - Size: Medium
   - Shape: Circular
   - Elevation: Convex
   - Texture: Smooth
   - Edges: Regular
2. **Milky White Colony**:
   - Size: Medium

- Shape: Circular (round edged)
- Elevation: Convex
- Surface: Mucoid
- Edges: Regular

3. **Pink Colony**:
   - Size: Large
   - Shape: Circular
   - Elevation: Flat
   - Surface: Mucoid
   - Edges: Regular (irregular)
   - Cell Shape: Bacilli
   - Gram Stain: Positive

Table 5. Morphology of sugarcane root bacterial colonies week 3.

ISOLATE CODE	COLONY MACROSCOPY					
	Colony Color	**Size**	**Colony Form**	**Elevation**	**Surface**	**Edge**
1.	Yellow	Big	Irregular	Flat	Mucoid	Regular
2.	Pink	Big	Irregular	Flat	Smooth	Regular
3.	Yellow	Medium	Irregular	Flat	Smooth	Regular
4.	Blue in the center	Medium	Irregular	Convex	Smooth	Irregular

Table 6. Morphology of sugarcane root bacterial cells week 3.

ISOLATE CODE	CELL MORPHOLOGY	
	Cell Shape	**Grams**
1.	Basil	Positive
2.	Basil	Positive
3.	Basil	Positive

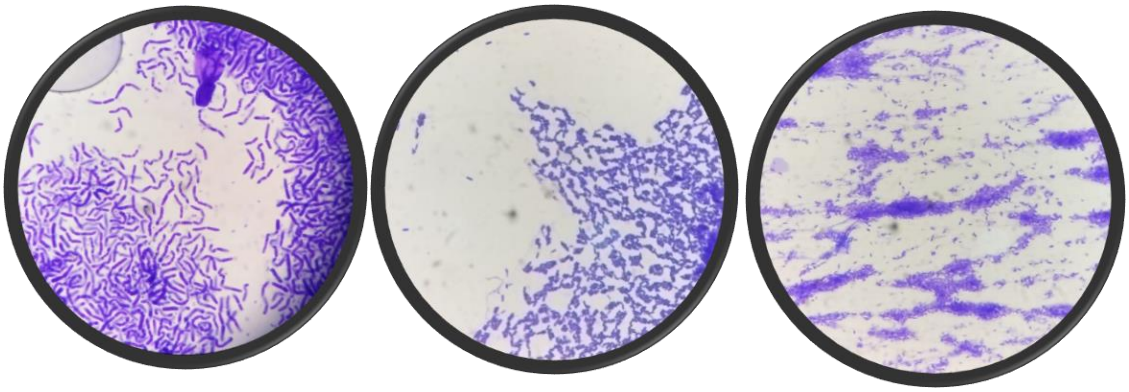
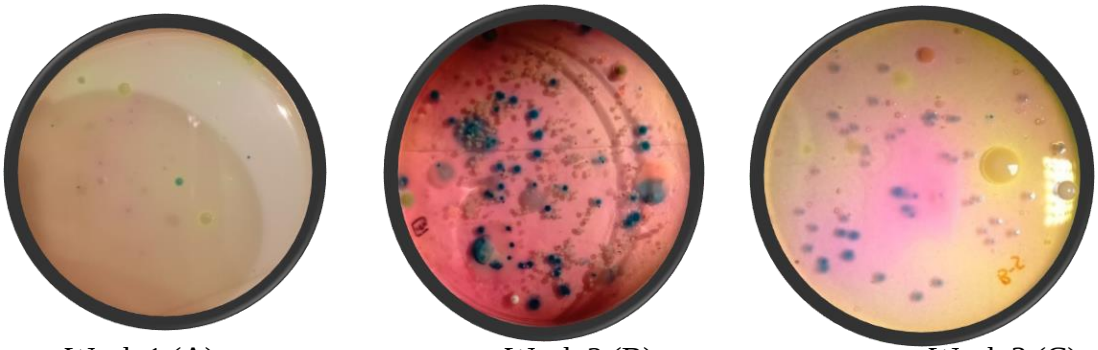


Figure 5. Results of gram staining of sugarcane root bacteria week 3.

Based on the morphological observations of sugarcane root bacterial colonies in week 3, four distinct colony colors were observed: yellow, pink, and blue. The characteristics of each colony are as follows:

1. **Yellow Colony (Large Size)**:
   - Shape: Irregular (not edged)
   - Elevation: Flat (even)
   - Surface: Mucoid
   - Edges: Regular
2. **Pink Colony (Large Size)**:
   - Shape: Irregular (not edged)
   - Elevation: Flat
   - Surface: Smooth
   - Edges: Regular
3. **Yellow Colony (Medium Size)**:
   - Shape: Irregular (not edged)
   - Elevation: Flat
   - Surface: Smooth
   - Edges: Regular
4. **Blue Colony (Medium Size, Blue Center)**:
   - Shape: Irregular (not edged)
   - Elevation: Convex
   - Surface: Smooth
   - Edges: Regular (irregular)
   - Cell Shape: Bacilli
   - Gram Stain: Positive



Week 1 (A) Week 2 (B) Week 3 (C)

Figure 6. Growth of bacterial colonies in sugar cane field exploration (A, B, C).

Table 7. Bacterial colony morphology of sugar cane field exploration week 1.

ISOLATE CODE	COLONY MACROSCOPY					
	Colony Color	**Size**	**Colony Form**	**Elevation**	**Surface**	**Edge**
1.	Yellow	Medium	Circular	Convex	Mucoid	Regular
2.	Blue	Medium	Circular	Flat	Smooth	Irregular
3.	Milk White	Medium	Circular	Convex	Smooth	Regular
4.	Pink	Medium	Irregular	Unboned	Smooth	Irregular
5.	Yellow	Medium	Irregular	Raised	Smooth	Irregular
6.	Blue	Pints	Circular	Convex	Smooth	Regular

Table 8. Cell morphology sugarcane field exploration bacteria week 1.

ISOLATE CODE	CELL MORPHOLOGY	
	Cell Shape	Grams
1.	Basil	Positive
2.	Basil	Positive
3.	Basil	Positive
4.	Basil	Positive
5.	Basil	Positive
6.	Basil	Positive

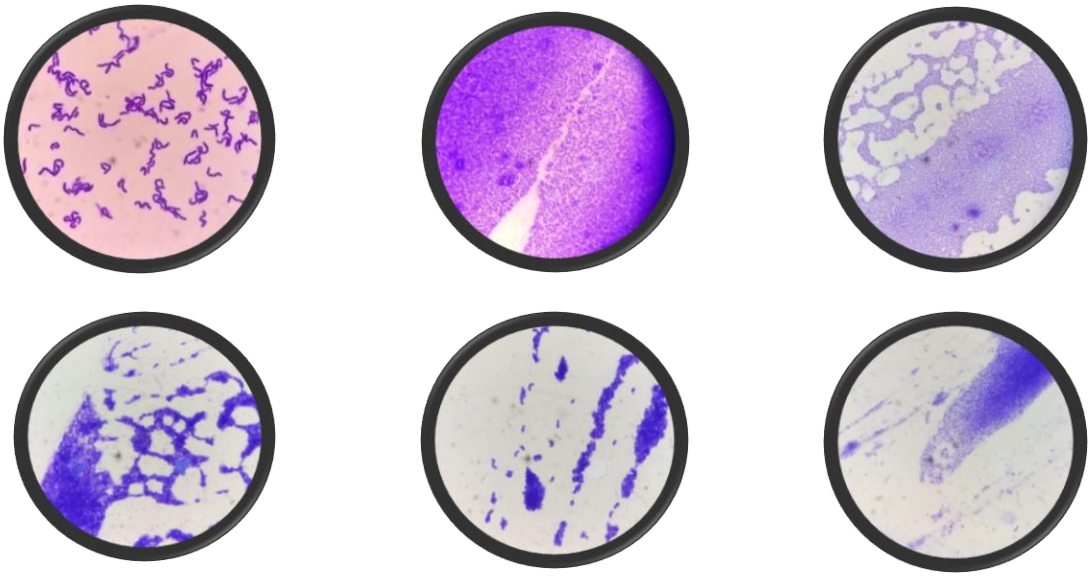


Figure 7. Results of bacterial gram staining of sugar cane field exploration week 1.

Based on the morphological observations of the colonies from sugarcane field exploration in the first week, six distinct colony colors were identified: yellow, blue, milky white, and pink. The characteristics of each colony are as follows:

1. **Yellow Colony** (Medium Size):
   - Shape: Circular
   - Elevation: Convex
   - Surface: Mucoid
   - Edges: Regular
2. **Blue Colony** (Medium Size):
   - Shape: Circular (round edged)
   - Elevation: Flat
   - Surface: Smooth
   - Edges: Irregular
3. **Milky White Colony** (Medium Size):
   - Shape: Circular (round edged)

- Elevation: Convex
- Surface: Smooth
- Edges: Regular

4. **Pink Colony** (Medium Size):
   - Shape: Irregular (not edged)
   - Elevation: Umbonate
   - Surface: Smooth
   - Edges: Irregular
   - Cell Shape: Bacilli
   - Gram Stain: Positive
5. **Yellow Colony** (Medium Size):
   - Shape: Irregular (not edged)
   - Elevation: Raised
   - Surface: Smooth
   - Edges: Irregular
   - Cell Shape: Bacilli
   - Gram Stain: Positive
6. **Blue Colony** (Pinpoint Size):
   - Shape: Circular (round edged)
   - Elevation: Convex
   - Surface: Smooth
   - Edges: Regular
   - Cell Shape: Bacilli
   - Gram Stain: Positive

Table 9. Bacterial colony morphology of sugarcane field exploration week 2.

ISOLATE CODE	COLONY MACROSCOPY					
	Colony Color	**Size**	**Colony Form**	**Elevation**	**Surface**	**Edge**
1.	Blue	Medium	Circular	Convex	Smooth	Regular
2.	Milk White	Medium	Circular	Convex	Mucoid	Regular
3.	Pink	Big	Irregular	Flat	Mucoid	Irregular

Table 10. Cell morphology sugarcane field exploration bacteria week 1.

ISOLATE CODE	CELL MORPHOLOGY	
	Cell Shape	**Grams**
1.	Basil	Positive
2.	Basil	Positive
3.	Basil	Positive

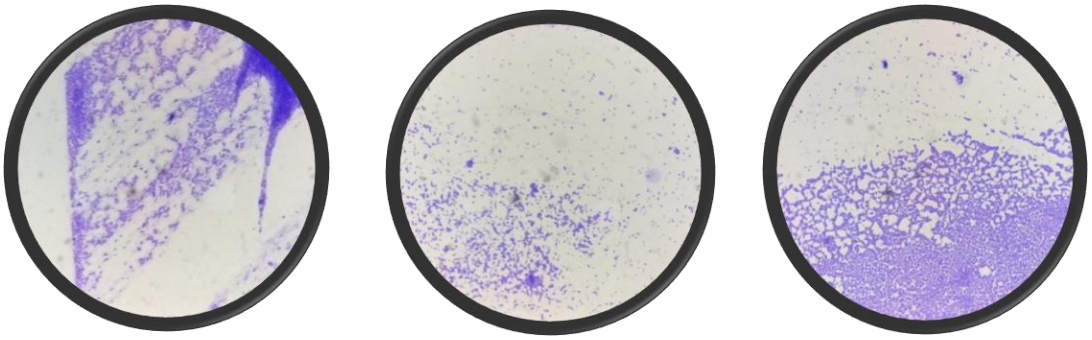


Figure 8. Results of bacterial gram staining of sugar cane field exploration week 2.

Based on the morphological observations of the colonies from sugarcane field exploration in the second week, three distinct colony colors were identified: blue, milky white, and pink. The characteristics of each colony are as follows:

1. **Blue Colony** (Medium Size):
   - Shape: Circular
   - Elevation: Convex
   - Surface: Smooth
   - Edges: Regular
2. **Milky White Colony** (Medium Size):
   - Shape: Circular (round edged)
   - Elevation: Convex
   - Surface: Mucoid
   - Edges: Regular
3. **Pink Colony** (Large Size):
   - Shape: Irregular
   - Elevation: Flat
   - Surface: Mucoid
   - Edges: Irregular

Table 11. Bacterial colony morphology of sugar cane field exploration week 3.

ISOLATE CODE	COLONY MACROSCOPY					
	Colony Color	**Size**	**Colony Form**	**Elevation**	**Surface**	**Edge**
1.	Blue Center	Small	Irregular	Flat	Smooth	Irregular
2.	Yellow	Big	Circular	Convex	Mucoid	Regular
3.	Pink	Big	Irregular	Flat	Smooth	Irregular
4.	Yellow	Medium	Irregular	Flat	Smooth	Irregular
5.	Pink	Small	Circular	Convex	Smooth	Regular

Table 12. Cell morphology sugarcane field exploration bacteria week 3.

ISOLATE CODE	CELL MORPHOLOGY	
	Cell Shape	Grams
1.	Basil	Positive
2.	Basil	Positive
3.	Basil	Positive
4.	Basil	Positive
5.	Basil	Positive

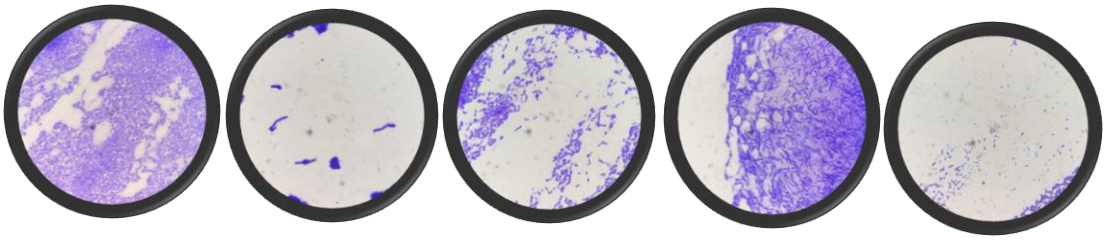


Figure 9. Results of bacterial gram staining on sugar cane field exploration week 3.

Based on the morphological observations of the colonies from sugarcane field exploration in week 3, five distinct colony colors were identified: blue, yellow, pink, and another yellow. The characteristics of each colony are as follows:

1. **Blue Colony** (Small Size, Blue Center):
   - Shape: Irregular
   - Elevation: Flat
   - Surface: Smooth
   - Edges: Irregular
2. **Yellow Colony** (Large Size):
   - Shape: Circular (round edged)
   - Elevation: Convex
   - Surface: Mucoid
   - Edges: Regular
3. **Pink Colony** (Large Size):
   - Shape: Irregular
   - Elevation: Flat
   - Surface: Smooth
   - Edges: Irregular
4. **Yellow Colony** (Medium Size):
   - Shape: Irregular
   - Elevation: Flat
   - Surface: Smooth
   - Edges: Irregular
5. **Pink Colony** (Small Size):
   - Shape: Circular
   - Elevation: Convex

- Surface: Smooth
- Edges: Regular

CONCLUSION

The growth of sugarcane root bacterial colonies increased significantly over the three-week period in the fermented liquid. In week 1, the number of colonies was 7.7×10^7 CFU/ml, which increased to 28.8×10^7 CFU/ml in week 2 and further to 58×10^7 CFU/ml in week 3. Similarly, the exploratory bacterial colonies from sugarcane land also showed substantial growth, with 3.4×10^7 CFU/ml in week 1, increasing to 19.7×10^7 CFU/ml in week 2 and further to 62×10^7 CFU/ml in week 3 in the fermented liquid.

The morphology of sugarcane root bacterial colonies exhibited various characteristics from week 1 to week 3, including different colony colors such as yellow, pink, blue, milky white, and blue center. The colony sizes ranged from small to large, with shapes varying from circular to irregular. Elevation was observed as convex or flat, and the surface texture ranged from smooth to mucoid. The edges of the colonies were irregular or regular, and the bacterial cell shapes were mainly bacilli and gram-positive.

Similarly, the bacterial colony morphology observed in sugarcane field exploration from week 1 to week 3 showed a diverse range of characteristics. Colony colors included yellow, pink, blue, milky white, and blue center. Colony sizes varied from small to large, with shapes ranging from circular to irregular. Elevation was observed as convex, flat, unbonate, or raised, and the surface texture ranged from smooth to mucoid. The edges of the colonies were irregular or regular, and the bacterial cell shapes were predominantly bacilli and gram-positive. Overall, the morphological observations provide valuable insights into the diversity and characteristics of sugarcane root and field bacterial colonies over time.

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